Role of mucosal prostaglandins and DNA synthesis in gastric cytoprotection by luminal epidermal growth factor

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SUMMARY This study compares the effect of epidermal growth factor and prostaglandins (PGE$_2$ or PGI$_2$), applied topically to gastric mucosa, on gastric secretion and formation of ASA-induced gastric ulcerations in rats. Epidermal growth factor given topically in non-antisecretory doses prevented dose-dependently the formation of ASA-induced ulcers without affecting prostaglandin generation but with a significant rise in DNA synthesis in the oxyntic mucosa. The anti-ulcer effect of topical prostaglandins was also accompanied by an increase in DNA synthesis. This study indicates that topical epidermal growth factor, like PGE$_2$ or PGI$_2$, is cytoprotective and that this cytoprotection is not mediated by the inhibition of gastric secretion or prostaglandin formation but related to the increase in DNA synthesis in oxyntic mucosa.

Epidermal growth factor (EGF) is a single chain of 53 amino acid residues which inhibits gastric acid secretion and stimulates epithelial cell proliferation.$^{1,2}$ A hypothetical role for EGF is protection of the gastrointestinal mucosa, as it is produced by salivary and duodenal glands$^3$ and may be secreted into the gut lumen. So far only prostaglandins (PGs) have been thought to be capable of protecting the mucosa against chemical or thermal injury and this property, termed 'cytoprotection', has been regarded as a unique feature of PGs.$^{4,5}$

This study was designed to compare the gastric secretory and cytoprotective effects of EGF and PGs applied topically to the gastric mucosa against aspirin-(ASA) induced mucosal lesions and to elucidate the possible role of PG formation and DNA synthesis in the action of EGF on gastric mucosa.

METHODS

Wistar rats, weighing 150-200 g, were used for the studies on gastric secretion, gastric ulcer production, prostaglandin generation, and DNA synthesis in the oxyntic mucosa.

SECRETORY STUDIES

Ten rats were prepared with chronic gastric fistulae, as described by Lane and Ivy,$^6$ and used for the secretory studies. Experiments started about one month after surgery. Rats were allowed only water for at least 24 hours and were kept in individual metabolic cages to prevent coprophagia. In the morning, animals were placed in restraining Bollman cages, the gastric fistula was opened, and the stomach washed out with tap-water. The tests were conducted with seven to 10 day intervals between successive experiments on the same animal. Gastric secretion was collected at half-hourly intervals in the graduated tubes, the volume was noted, and hydrogen ion concentration was determined by titration to pH 7.0 with 0.1 N NaOH using an automatic titrator (Radiometer, Copenhagen) and ion output was expressed in mmol/30 min. Pepsin content was also measured in each sample using Anson's haemoglobin method$^7$ and expressed in mg/30 min.

Two series of secretory studies were performed, one under basal conditions and another after pentagastrin stimulation. Basal gastric secretion...
was collected during three 30 minute periods and
then EGF, PGE₂, or PG₁₂ was instilled into
the stomach in a dose of 100 μg/kg and the gastric-
fistula was closed for 25 minutes. After this inter-
ruption, the fistula was opened again, the stomach
drained for five minutes, and this collection dis-
carded. The collection was then continued for
another 90 minutes with saline infused subcutane-
ously at a rate of 4 ml·h throughout. In another
series of tests, pentagastrin was infused subcutane-
ously at a rate of 200 μg/kg per h throughout the
experiment. The collection of gastric juice and the
administration of EGF, PGE₂, or PG₁₂ was similar
to the tests with basal secretion. All secretory
tests were performed on the same 10 gastric fistula
rats.

**Production of Gastric Ulcers**

Gastric mucosal lesions were produced by in-
tra gastric instillation of aspirin (ASA) plus 0.15 M
HCl as described by Kauffman and Grossman.³
For this purpose, rats were surgically prepared with
a polyethylene tube in the stomach about two hours
before the experiment. The intra gastric instillation
of ASA as a bolus injection of 60 mg kg was followed
by a constant infusion of 42 mg kg/h for three
hours. A simultaneous gastric perfusion with 0.15
M HCl was carried out at a rate of 4 ml·h. In tests
with epidermal growth factor or prostaglandins,
the solution of these compounds was injected
intragastrically 30 minutes before and continued
throughout ASA administration. At the end of the
experiment, the animals were killed, the stomach
was removed and opened along the greater curvature.

The mucosa was quickly examined, the surface area
of ulcers was measured planimetrically (mm²) and
the mucosal biopsy samples were taken for deter-
minations of PG and DNA.

Studies were performed on groups of eight to
30 rats, fasted 24 hours, and given one of the follow-
ing intragastric treatments: (1) saline (con-
trol); (2) epidermal growth factor; (3) PGE₂; (4)
PG₁₂; (5) ASA + HCl; (6) ASA - HCl - EGF; (7)
ASA - HCl - PGE₂; and (8) ASA - HCl - PG₁₂.
As the animals receiving combinations 1-4 did not
show ulceration, their stomachs were used only for
the prostaglandin generation and DNA synthesis.
In rats with five to eight treatments the mean
ulcer area was also measured.

Solutions of epidermal growth factor and prosta-
glandins were freshly prepared before the exper-
iment. EGF or PGE₂ was dissolved in saline and
kept at room temperature, whereas PG₁₂ was
dissolved in isotonic Tris buffer (pH 9.6) and kept
in ice throughout the infusion. Epidermal growth
factor was isolated from mouse submaxillary
glands according to the method of Savage and
Cohen⁶ with minor modifications. The product, a
single peak on Bio Gel P-6, had a correct amino
acid ratio and was homogenous on acrylamide
gel electrophoresis. It strongly inhibited histamine
stimulated acid secretion from the dog Heidenhain
pouch and was equipotent with highly purified
urogastrone. Both prostaglandins were a gift from
Dr J Griffith of Upjohn Co (Kalamazoo, Michigan).
Epidermal growth factor was given in a bolus
intra gastric injection of 1, 10, or 100 μg/kg and then
infused intragastrically in a supporting, 10 times
smaller dose (0.1, 1, or 10 μg/kg·h respectively) for
3.5 hours either alone or in combination with
acidified ASA added to the infusion 30 minutes
after the start of EGF administration. PGE₂ or
PG₁₂ was given first as an intragastric bolus injec-
tion of 100 μg/kg and this was continued by
intragastric infusion of 10 μg/kg·h for 3.5 hours
either alone or in combination with ASA as in tests
with EGF.

**Measurement of Mucosal Generation of PGS**

Assessment of the capacity of oxyntic mucosa to
generate prostaglandins was determined by the
method of Whittle.¹⁰ Generation was measured by a
quick extraction and immediate bioassay, PGE₂
and PGF₂α by using superfused biological detectors
—that is, strips of rat stomach and colon¹¹—and
PG₁₂ by using its anti-aggregatory activity in
rabbit platelet-rich plasma.¹² The amounts of the
generated PG were expressed in ng·g tissue weight.

**Determination of DNA Synthesis**

DNA synthesis in the oxyntic mucosa was measured
by in vitro incubation of fundic mucosa in Eagle's
minimal essential culture medium containing 2
μCi ml⁻¹[H]-thymidine (Amersham, England) as
described previously.¹³ The incorporation of [³H]-
thymidine into DNA was determined by counting
0.5 ml of the DNA-containing filtrate in a Beckman
liquid scintillation counting system. Results were
calculated as disintegrations per min (DPM) per mg
of wet weight and expressed as DPM per μg DNA.
The DNA of the samples was determined by the
Burton procedure modified by Giles and Myers.¹⁴

Plasma salicylate levels in animals treated with
intra gastric ASA were measured in the blood
samples taken from the inferior vena cava at the end
of the experiment using Saltzman's method.¹⁶

All values reported are the means (± SEM).
These means were used in the t test for paired
values to evaluate the significance of differences in
gastric secretory outputs, ulcer area, prostaglandin
generation, and DNA synthesis in gastric mucosa.
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between control groups and treated with EGF, PGE₂, or PGI₂. As used in the text, significance indicates \( p < 0.05 \).

Results

EFFECT OF EGF AND PGS ON GASTRIC SECRETION

Figures 1 and 2 show the effects of epidermal growth factor in a dose of 100 \( \mu g/kg \) on acid and pepsin secretion. This factor did not significantly affect gastric secretion either under basal conditions or after pentagastrin stimulation. PGE₂ and PGI₂ given intragastrically in a dose of 100 \( \mu g/kg \) did not influence gastric acid or pepsin secretion and these results were omitted from the presentation.

EFFECT OF EGF AND PGS ON PG GENERATION AND DNA SYNTHESIS IN INTACT RATS

In rats given only intragastric saline, the PGE₂ and PGI₂ were generated in the oxyntic mucosa in amounts of about 446 ± 148 and 271 ± 129 ng/g, respectively (Fig. 3). The generation of PGF₂ was negligible and the data were not included. DNA synthesis in the intact mucosa was about 56 ± 4 DPM \([^{3}H]\)-thymidine/μg DNA and this was accepted as 100% control. Epidermal growth factor instilled intragastrically in a dose of 100 \( \mu g/kg \) followed by intragastric infusion of 10 \( \mu g/\) kg/h for 3.5 hours did not significantly affect the generation of PGE₂ or PGI₂ but increased DNA synthesis in oxyntic mucosa by approximately 35% (Fig. 4). When a test infusion of 100 \( \mu g/kg \) PGE₂ or PGI₂ was given intragastrically the generation of prostaglandins was not determined because the samples were contaminated with exogenous PGs. DNA synthesis was not affected by this treatment of oxyntic mucosa and the data were omitted from the presentation.

![Fig. 1](image1)

*Fig. 1* Effect of intragastric administration of EGF (hatched) or saline (control) (dotted lines) on basal and pentagastrin stimulated acid secretion from 10 gastric fistula rats. Mean ± SEM of 10 experiments.

![Fig. 2](image2)

*Fig. 2* Pepsin outputs in test as in Fig. 1. (Key as in Fig. 1.)
EFFECTS OF EGF AND PGS ON GaSTRIC ULCER FORMATION, PG GENERATION, AND DNA SYNTHESIS IN ASA-TREATED RATS

All 30 control rats treated with intragastric instillation of ASA plus HCl developed gastric ulcers that occurred mainly in the oxyntic gland area—the mean ulcer area was 7.65±0.91 mm² (Fig. 5). Epidermal growth factor given intragastrically 30 minutes before and throughout intragastric infusion of acidified ASA caused a dose dependent reduction in the mean ulcer area. This reduction in the ulcer area by EGF at doses of 1, 10, and 100 μg/kg was about 55%, 72%, and 85%, respectively. PGE₂ or PG₁₂ in a dose of 100 μg/kg caused similar reduction in the ulcer area to that achieved by EGF in a dose of 100 μg/kg.

ASA instilled intragastrically in combination with HCl reduced the generation of PGE₂ in oxyntic mucosa by about 80%, and caused the almost complete disappearance of PG₁₂. The generation of prostaglandins in animals treated with a combination of acidified ASA plus epidermal growth factor in a dose of 100 μg/kg was not significantly different from that observed in animals receiving ASA alone (Fig. 3).

The effects of EGF, PG₂, or PG₁₂, all given intragastrically in a bolus dose of 100 μg/kg, followed by infusion of 10 μg/kg for 3-5 hours, on DNA synthesis in ASA-treated rats are shown in Fig. 4. The intragastric administration of ASA plus HCl, which caused the formation of mucosal lesions, significantly depressed DNA synthesis by

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**Fig. 3** Generation of PGE₂ and PG₁₂ in oxyntic mucosa of intact rats infused intragastrically with saline, epidermal growth factor, acidified ASA alone, or in combination with epidermal growth factor. Each column represents mean ± SEM of results from eight to 30 rats.

**Fig. 4** DNA synthesis in oxyntic mucosa of rats treated with topical epidermal growth factor alone and acidified ASA alone or in combination with EGF, PGE₂, or PG₁₂. Each column represents mean ± SEM of results from eight to 30 rats. *Significant (P < 0.5) increase above control level. **Significant decrease below the control level. ***Significant increase above the level found in rats treated with acidified ASA.

**Fig. 5** Mean ulcer area in animals treated with intragastric acidified ASA alone or in combination with epidermal growth factor, PGE₂, or PG₁₂. Each column represents mean ± SEM of results from eight to 30 animals. *Significant decrease below the value obtained in rats treated with acidified ASA.
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about 28%. Pretreatment with EGF, PGI₂, or PGE₂ significantly raised DNA synthesis in the gastric mucosa to a level similar to that observed in control animals.

Plasma salicylate levels in rats receiving only intragastric ASA plus HCl averaged 396 ± 47 μg/ml and it was not significantly different in animals receiving epidermal growth factor or prostaglandins. These data are not included for the sake of clarity.

Discussion

The results show that epidermal growth factor is capable of preventing ASA-induced gastric ulcerations and the effect is not related to inhibition of gastric secretion or prostaglandin generation but to DNA synthesis in oxyntic mucosa.

The formation of gastric lesions has been attributed to a mucosal deficiency of prostaglandins because of the known irreversible inactivation of the PG synthetase system by ASA. This notion is supported by our findings that ASA almost completely suppresses the generation of prostaglandins in oxyntic mucosa and that PGE₂ and PGI₂, which are the major products of the biotransformation of arachidonate via the cyclo-oxygenase pathway in the rat gastric mucosa, are capable of preventing the development of ASA-induced ulcers. The protective effects of exogenous PGE₂ and PGI₂ against ASA-induced mucosal lesions could be explained by the replenishment of the mucosal level of these compounds. Thus, normal generation of mucosal PGE₂ and PGI₂ appears essential for the protection of gastric mucosa against chemical injury by ASA. This action of prostaglandins does not appear to be mediated through the inhibition of gastric secretion (cytoprotection) as natural prostaglandins were used topically and these were found to be without any influence on basal or pentagastrin-induced gastric acid or pepsin secretion.

Although gastric cytoprotection was claimed to be a unique property of prostaglandins, our study provides evidence that epidermal growth factor is also cytoprotective when applied topically in a dose which does not affect gastric secretion. It appears that topical EGF neither affects the prostaglandin generation in the intact mucosa nor prevents the suppression of this generation in ASA-treated animals. This rules out the possible mediation of mucosal prostaglandins in the cytoprotective action of EGF.

The major finding is that pretreatment with acidified ASA decreases DNA synthesis with gastric ulceration and EGF reverses this change in DNA synthesis. The reason for the reduction in DNA synthesis by ASA is unknown but it may be due to destruction of large numbers of mucosal cells, possibly because of the removal by ASA of the cytoprotective function of mucosal prostaglandins. Indeed, topical administration of exogenous prostaglandins in a non-antisecretory dose prevents the fall in DNA synthesis while protecting the mucosa against ulcer formation. Thus, the common action of both epidermal growth factor and prostaglandins is the prevention of DNA synthesis which renders the oxyntic mucosa more resistant to the ulcerogenic effect of acidified ASA.

It may be worthwhile to mention that a similar reduction in DNA synthesis in the gastric mucosa of animals with stress-induced gastric ulcerations has been reported. The application of trophic substances such as growth hormone or pentagastrin prevented the formation of these lesions and this effect was also closely correlated with an increase in DNA synthesis.

This study demonstrates that epidermal growth factor is cytoprotective after topical application to the gastric mucosa when the inhibitory effect of the peptides is entirely excluded. This indicates that the antisecretory and growth-promoting actions of EGF are two independent features of this peptide. As EGF is produced by the salivary and duodenal glands and may be released into the gut lumen, it may serve as a natural growth-promoting factor for the mucosa, and is also responsible for its protection against injury by various noxious agents. Thus, the gastrointestinal mucosa is provided with at least two separate cytoprotective mechanisms, one related to the local generation of prostaglandins and another involving the release and action of epidermal growth factor.

References

Konturek, Brzozowski, Piastucki, Dembinski, Radecki, Dembinska-Kiec, Zmuda, and Gregory


